THE UNIVERSITY OF ALBERTA

THE EFFECTS OF EXERCISE UPON THE SKELETAL MUSCLE GLYCOGEN STORES OF ACTIVE AND SEDENTARY SUBJECTS

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "The Effects of Exercise upon the Skeletal Muscle Glycogen Stores of Active and Sedentary Subjects," submitted by Ronald Sidney Lappage in partial fulfilment of the requirements for the degree of Master of Arts.

ABSTRACT

The glycogen levels of working skeletal muscle and the adaptations of the muscle in a trained and untrained state to maximal and submaximal work loads were examined.

Significant changes ($p < 0.05$) for all groups from initial to fatigue glycogen levels were observed on the submaximal test. However, no significant differences were noted between the fatigue and final-resting values. For the initial values a significant difference ($p < 0.05$) existed only between the sedentary and active post-training groups, but there was a trend of progressive decrease with the active post-training group having the highest value. For the maximal test only the active post-training and sedentary groups had a significant change ($p < 0.05$) in muscle glycogen levels from initial to fatigue values. The other 2 groups experienced small decreases. A large significant difference was observed between the initial glycogen levels of the sedentary and active post-training groups. The initial values for the other 2 groups were almost identical, and they differed significantly from the sedentary and active post-training groups. As for the submaximal test, generally no significant differences were seen between the fatigue and final-resting values. All 4 curves for the submaximal and maximal work loads respectively were similarly shaped. Muscle glycogen levels at the point of fatigue were lower for the submaximal than for the maximal test but still not as low as reported in other literature.

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CHAPTER I

INTRODUCTION

The body's main sources of glycogen, which is an indirect source of energy for muscular work, are found in the liver and muscles. During exercise, when extra energy is required, the glycogen stored in the muscle is broken down under the influence of the enzyme phosphorylase-a into *glucose-1-P and then continues to follow the many reactions of the glycolytic pathway until pyruvate is formed.

*The abbreviations used in this Thesis are as follows:

Abbreviation	Meaning
glucose-6-P	glucose - 6 - phosphate
glucose-1-P	glucose - 1 - phosphate
glucose-U-C^{14}	glucose - uridine - carbon 14
ATP	adenosine triphosphate
UTP	uridine - 5 - triphosphate
UDPG	uridine diphosphoglucose
NAD	nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
glycogen synthetase	uridine diphosphoglucose-glycogen-transglucylase
branching enzyme	amylo (1, 4→1, 6) transglucosylase
debranching enzyme	amylo - 1, 6 - glucosidase
PWC 170	physical work capacity at a heart rate of 170 b.p.m.
PP	pyrophosphate
Pi	inorganic phosphate

If the work is aerobic the pyruvate enters the Krebs cycle which results in the formation of CO_2, H_2O and ATP which is broken down to provide the source of energy for muscular contraction. If the work is anaerobic the pyruvate is converted to lactate which accumulates in the muscles and plasma.

Previously, in order to study muscle glycogen, it was necessary to study animals, cadavers, and amputated limbs (23:11), but this proved to be an inadequate method since glycogen is rapidly broken down by cellular enzymes. However, the development of the muscle biopsy technique has enabled researchers to follow glycogen metabolism in human skeletal muscles.

Effects of Exercise on Muscle Glycogen Levels

Most research conducted on the effects of exercise on glycogen levels has been carried out using a submaximal work load whereby the subjects have worked for a period of 1 to 3 hours before fatiguing. Saltin (39) has suggested that the available energy stores of carbohydrates limit the individual's capacity for prolonged work. He has found a large initial decrease in muscle glycogen levels during the first 20 minutes of submaximal work (71% to 85%) followed by a moderate decrease in glycogen and finally, a slower decrease. A high correlation was found between the near absence of glycogen and the onset of fatigue, and thus Saltin (39) has concluded that the ability to perform very heavy prolonged exercise was directly related to the glycogen stores of the body. It has been further observed that a rapid initial increase in blood lactate, (in about the first 20 minutes of exercise) followed by a leveling off of the lactate level occurred, and then a decrease in lactate as exercise was

prolonged.

Hultman (24) has found a similarly large decrease in muscle glycogen during the first 15 minutes of work and has attributed this in part to the formation of lactic acid, which was most noticeable at this time. A more pronounced decrease in muscle glycogen and a greater increase in lactate in the untrained group as opposed to the trained during the first 15 minutes was also evident, but this difference was found only in the first work period. Muscular work still continued for some minutes when the muscle glycogen content was practically zero. In subjects working at 60% of PWC 170, the glycogen store decreased about 80% and a high correlation was found between the initial glycogen consumption in individual subjects and work performance time. In a non-working muscle during submaximal work, no decrease in the concentration of glycogen has been found.

There appears to be a dearth of research considering muscle glycogen levels at maximal work loads; that is, when the subject has worked for only 3 to 7 minutes before fatigue. Consequently, no literature has indicated what happens to the glycogen level after a short period of maximal work.

Statement of the Problem

The purpose of this thesis was to examine the glycogen levels of skeletal muscles and the adaptations of the muscles in a trained and untrained state to maximal and submaximal work loads.

Rationale behind the Study

Hultman et al (23) have used the muscle biopsy technique to study the influence of diet, exercise and fructose and glucose infusion upon muscle glycogen metabolism in humans, but have not yet noted the results of a training program upon the muscle glycogen levels before, during and after exercise. Procter and Best (35) confined their study of training effects on muscle glycogen to dogs and noted an optimum pre-exercise level of glycogen which was not surpassed by continued training. However, they did not examine the glycogen levels during a final post-training exercise session. The muscle glycogen levels before and immediately after a short maximal work effort have not been investigated.

Therefore, a study was necessary to follow the muscle glycogen levels in humans before, during, and after a prolonged exercise as well as before and after a short maximal work period. A comparison of these results could be made, and the differences created by a training program upon a select group of subjects could also be observed. A comparison between the effects of a fatiguing prolonged exercise period and a fatiguing short high work level exercise period could show whether or not the glycogen level is depleted equally under both circumstances. After the training program it could be further noted whether training influences the initial, and post exercise glycogen levels for both types of exercise.

CHAPTER II

REVIEW OF THE LITERATURE

Glucose ($C_6H_{12}O_6$) is one of the monosaccharides belonging to the classes of hexoses (44:300). Glycogen is a polymeric storage form of carbohydrate and is found mostly in the liver and muscles. It can be derived from four distinct sources: monosaccharides, lactic and pyruvic acids, fats and amino acids (44:302) while glucose is derived by the digestion of polysaccharides and disaccharides.

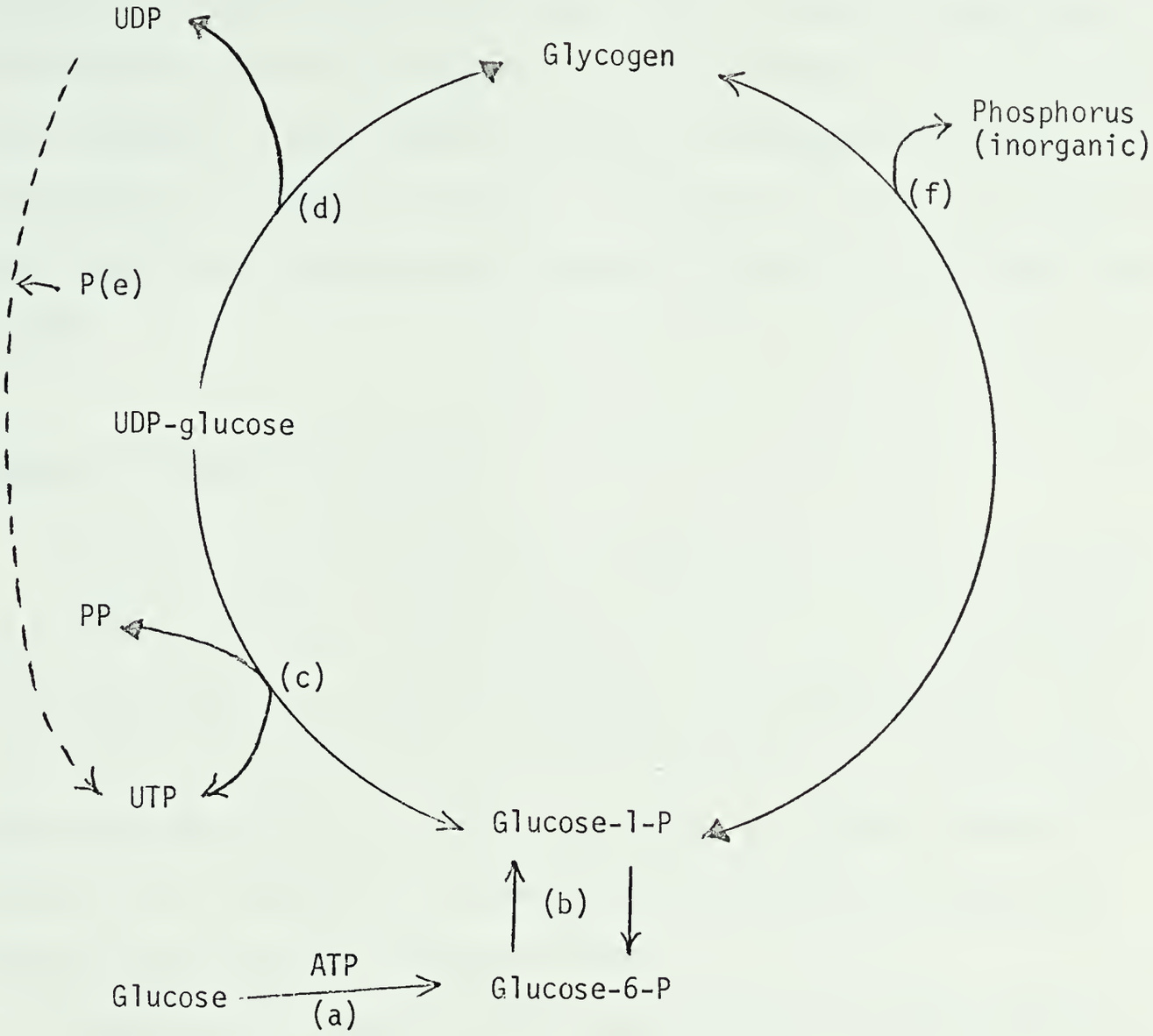
Glucose is absorbed and carried by the portal vein to the liver, its function being to supply energy for muscle and gland activity and for body heat. Carbohydrates are the materials most directly and most readily utilized by muscles and other structures for supplying the energy needed for their work. The level of blood sugar, 8 to 12 hours after a meal, of about 70 to 90 mg % is always present in the blood and available for immediate use (46:425). As this quantity is gradually reduced by the expenditure of energy, it is replenished by the uptake of food. However, since most persons eat only 2 or 3 meals per day, the sugar concentration may mount during the height of absorption to 130 mg % causing a temporary condition of hyperglycemia (44:301). Such a concentration of sugar would greatly increase the specific gravity of the blood and raise its osmotic pressure. The former would require more work by the heart; the latter would draw on excessive amounts of water from the tissues. To prevent this, the excess sugar (beyond 100 mg %) is withdrawn from the blood being stored in muscles and the liver as glycogen or being transformed into storage fat (44:301).

The Biochemical Mechanisms

Glycose ⇌ Glycogen

Glycogenesis is an intracellular process; therefore, glucose must move from the extracellular fluid through the cell membrane into the intracellular fluid. Active transport is involved in moving glucose into cells, and the hormone insulin facilitates this process. Potassium and phosphate ions are transported into cells simultaneously with glucose (4:437). Upon reaching the interior of the cells, glucose is first converted to glucose-6-phosphate by the action of the enzyme hexokinase and ATP. This reaction is essentially irreversible, although another enzyme, glucose-6-phosphatase, catalyzes the hydrolysis of glucose-6-P to free glucose with inorganic P, serving as a mechanism for the release of glucose from the liver to the blood (21:336). Glucose-6-P is converted to glucose-1-P by the action of phosphoglucomutase in the presence of catalytic amounts of glucose 1, 6-diphosphate. The glucose-1-phosphate is transformed to glycogen by reacting with uridine triphosphate (UTP) to form an active nucleotide, uridine diphosphate glucose (UDPG). Activated glucose molecules (UDPG) unite in the presence of glycogen synthetase and a branching enzyme to form glycogen (44:303). When the need for glucose arises, the glycogen is broken down to glucose-1-phosphate which is reconverted to glucose-6-phosphate by phosphoglucomutase. The conversion of this compound to glucose by glucose-6-phosphatase can occur only in the liver because liver cells contain the glucose-6-phosphatase enzyme necessary for catalyzing this reaction.

GLYCOGEN SYNTHESIS AND BREAKDOWN (41)



Pathways of the synthesis and breakdown of the α - 1, 4' linkages of glycogen (41).

The solid arrow heads indicate the probable preponderant direction of the reactions.

a - hexokinase
b - phosphoglucomutase
c - UDPG-pyrophosphorylase
d - glycogen synthetase and branching enzyme
e - UDP-kinase
f - phosphorylase

Therefore, in view of the muscle cells' lack of glucose-6-phosphatase no glucose can exist in the muscle (4:438). It should be further noted that glycogen in itself does not directly provide energy for work. It first must be converted via glucose-1-phosphate to glucose-6-phosphate which then follows the glycolytic pathway to provide energy in the form of ATP.

Control of Blood Glucose Level

The body maintains homeostosis of blood glucose from approximately 70 to 90 mg % (44:301). The main blood glucose homeostatic mechanisms are hormonal devices for controlling the rates of glycogenesis, glycogenolysis, glyconeogenesis and glucose metabolism by all body cells. These mechanisms are: the insulin mechanism, the adenohypophyseal-adrenal cortex mechanism, the sympathetic-adrenal medulla mechanism, and the thyroid hormone mechanism (4:439).

Insulin - An increase in blood glucose acts as a stimulus to the Beta cells of the islands of Langerhans in the pancreas, causing them to accelerate their secretion of insulin. The increased amount of insulin in the blood then speeds up glucose transport into cells from the extra-cellular fluid and results in blood glucose returning to normal. A decrease in blood glucose is followed by decreased insulin and glycogenesis (4:440).

Adenohypophyseal-adrenal cortex - This mechanism comes into function when the blood glucose falls below normal. Various kinds of physical and emotional stress situations are also postulated to activate this mechanism. Either a decrease in blood glucose or stress leads to

increased secretion of adrenocorticotrophic hormone (ACTH) by the adenohypophysis. ACTH then stimulates the adrenal cortex to speed up its secretion of glucocorticoids and finally, glucocorticoids accelerate gluconeogenesis (4:440).

Sympathetic-adrenal medulla - This mechanism is activated by hypoglycemia which is presumed to excite sympathetic centers in the hypothalamus. Increased epinephrine output by the adrenal medulla ensues. Epinephrine accelerates liver glycogenolysis and tends thereby to raise the blood glucose content (4:441).

Thyroid Hormone - The rate of glucose catabolism (glycolysis) and the Krebs cycle are partially controlled by the amount of thyroid hormone in the blood. An increase in thyroid hormone accelerates glycolysis as does an increase in insulin. It also may increase gluconeogenesis by accelerating catabolism of tissue proteins (4:441).

Role of the Liver

The liver maintains in the blood a fairly constant supply of glucose sufficient for immediate needs but not burdening the blood with an excessive load. Normally the liver converts glucose to glycogen for storage (glycogenesis) until the blood glucose level drops. When the tissues are using glucose at a rapid rate or when the organism is fasting, the blood sugar level drops, and stored glycogen in the liver is reconverted into glucose and is released into the blood (glycogenolysis) (44:301).

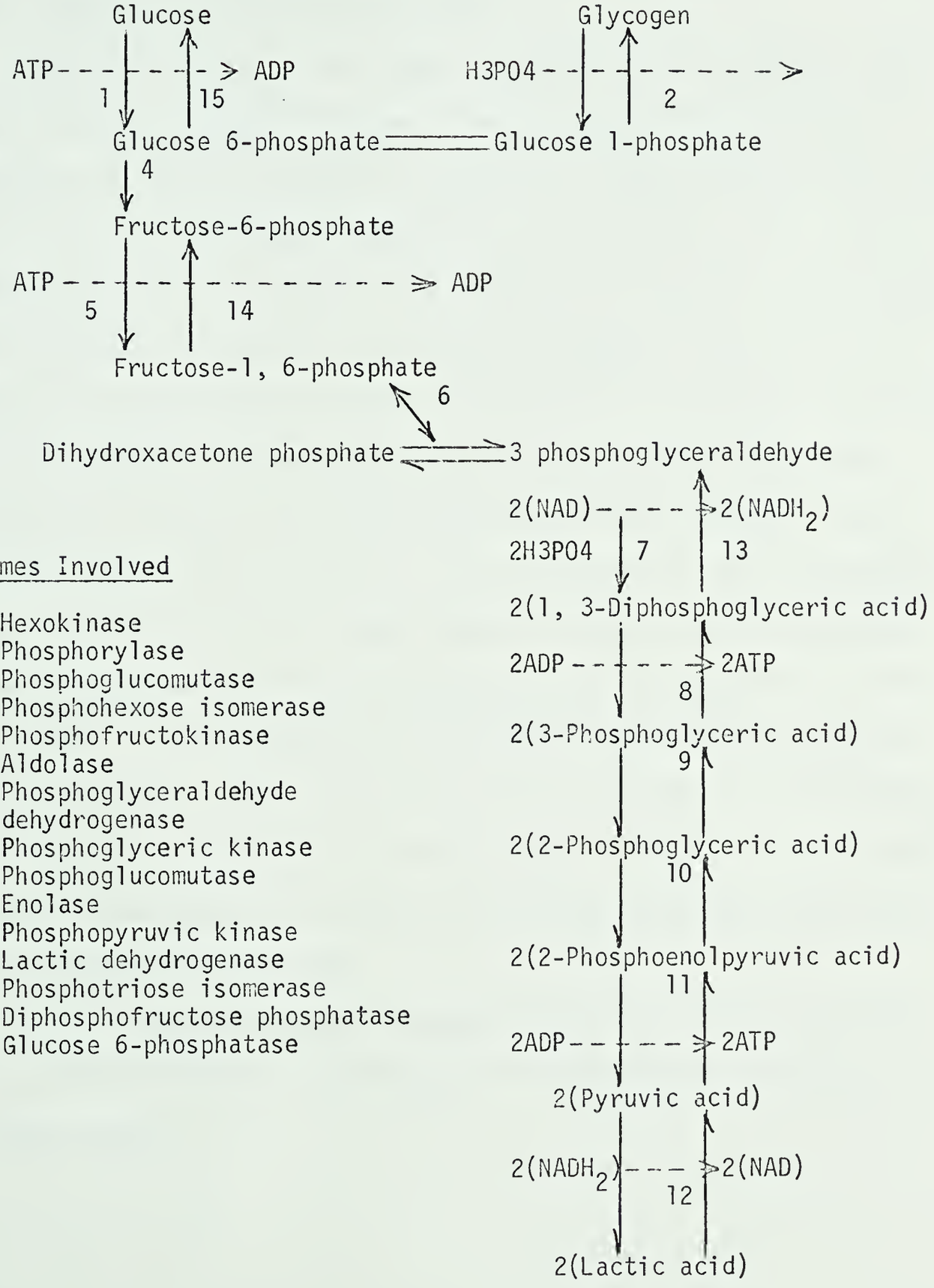
Glycolytic Pathway

It is generally agreed that the mechanical energy for muscular activity is provided by the chemical energy contained in the high energy organic phosphate compound, adenosinetriphosphate (ATP), which is split into adenosinediphosphate (ADP) and phosphate (P) (31:20). It was not until 1962 that a breakdown of ATP during muscular contraction was demonstrated by Cain and Davies (9) and Infante and Davies (27). During muscular exercise, the chemical processes of the muscles must provide a continuing supply of ATP to support the process of contraction. Two main pathways, one aerobic and one anaerobic, exist to reconstitute the supply of ATP. Under anaerobic conditions the glycolytic pathway utilizes carbohydrate exclusively and terminates in the production of lactic acid. On the other hand, the oxidative cycle can derive its initial substrate from either carbohydrate, fat, or protein.

As a result of glycolysis, one glucose molecule becomes two pyruvic molecules or two lactic acid molecules if oxygen is not plentiful in the cell. Concurrently, a small part of the energy in a glucose molecule is transferred to two molecules of adenosinetriphosphate (ATP), and the remainder is transformed into heat energy (4:43).

For the total glycolytic process, no net oxidation occurs and no oxygen is consumed (17:17). Thus, under conditions in which aerobic processes are not capable of producing an adequate supply of ATP, anaerobic metabolism becomes important. However, while no net oxidation occurs in the total pathway, the conversion of 3-phosphoglyceraldehyde to 1, 3 diphosphoglyceric acid does involve oxidation. This oxidation is balanced by a reduction of pyruvate to lactate. Oxidation and reduction

THE GLYCOLYTIC PATHWAY (17:17)



Enzymes Involved

1 Hexokinase
2 Phosphorylase
3 Phosphoglucomutase
4 Phosphohexose isomerase
5 Phosphofructokinase
6 Aldolase
7 Phosphoglyceraldehyde dehydrogenase
8 Phosphoglyceric kinase
9 Phosphoglucomutase
10 Enolase
11 Phosphopyruvic kinase
12 Lactic dehydrogenase
13 Phosphotriose isomerase
14 Diphosphofructose phosphatase
15 Glucose 6-phosphatase

take place by the transfer of electrons from 3-phosphoglyceraldehyde to nicotinamide adenine dinucleotide (NAD) and then from NAD^+ to pyruvate in the formation of lactic acid (17:17).

Glycogenesis from lactate occurs in the liver, but does not take place in skeletal muscle "in vivo" to any appreciable extent (19:9). A property of lactic acid which is important in the anaerobic production of ATP is its free diffusibility across cell walls (12, 33). This easy movement effects the removal of the metabolic end-product of glycolysis from within the cell which is particularly important since all of the intervening steps between glucose and lactic acid are freely reversible with the exception of the kinase reactions (31). Furthermore, the equilibrium of most of these reactions favours the production, rather than the degradation of glucose. The accumulation of any intermediate compound shifts the reaction back towards glucose. Of all the intermediary compounds, only lactate and pyruvate are freely diffusible out of the cell. It is important that substantial amounts of pyruvate be reduced to lactate rather than having pyruvate diffuse out of the cell. Loss of pyruvate prior to its reduction would eliminate it as an electron acceptor, resulting in an accumulation of $NADH_2$ which would also stop the glycolytic chain (17:18).

The lactate produced in the muscle during contraction is carried to the liver via the Cori cycle before it is converted via pyruvate to glycogen (19:9).

Krebs Cycle

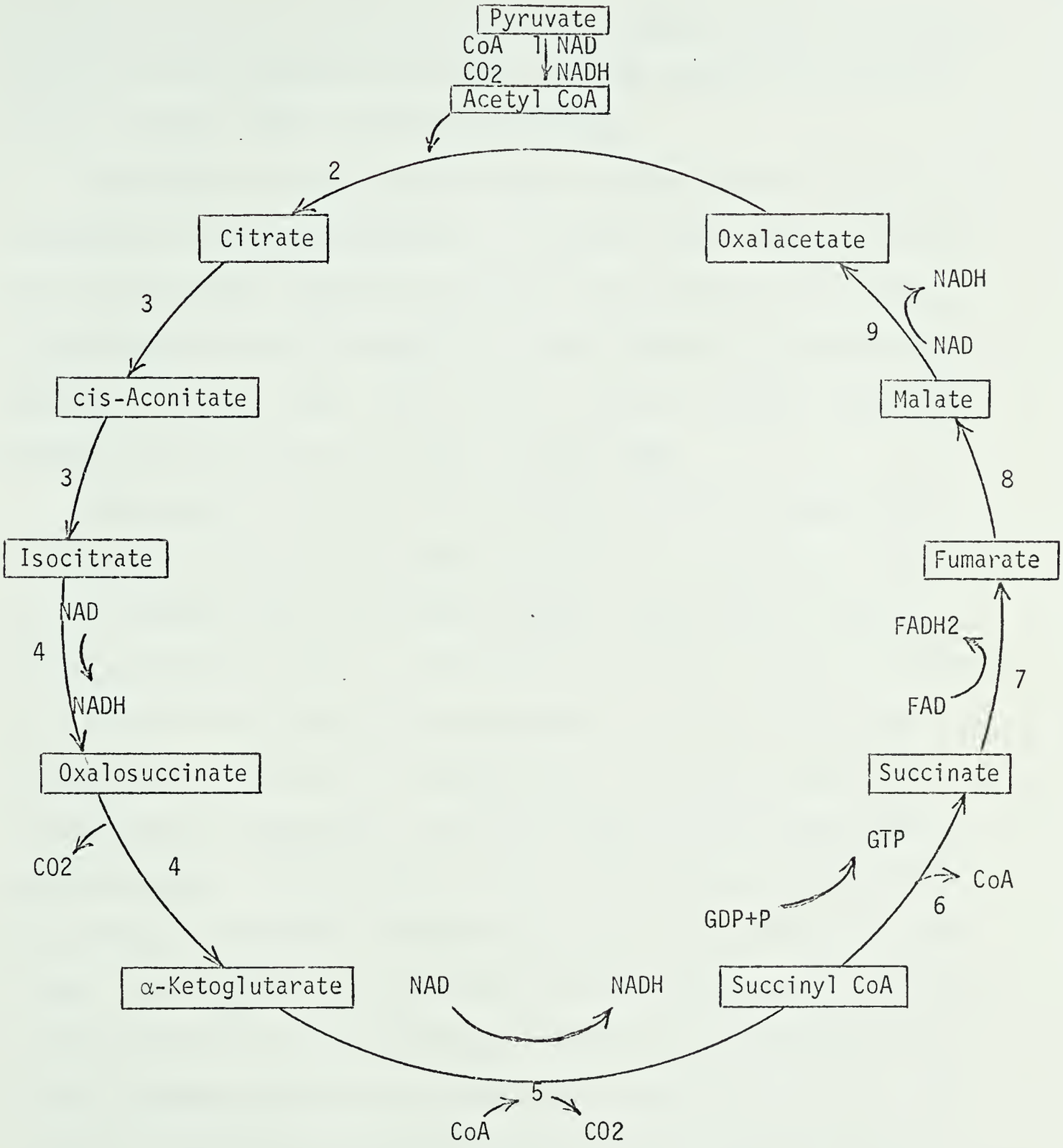
When adequate amounts of oxygen are present, pyruvic acid enters the mitochondria of the cell. Here, it combines with coenzyme A (4:44), and enters the Krebs cycle. In one complete cycle, 3 molecules of H_2O and 1 ATP are consumed, and in the process 2 CO_2, 8 H (electrons), 1 ATP, and oxoloacetic acid are generated. The most important function of the Krebs cycle is the generation of electrons which are passed on by means of NAD, NADP and flavoproteins to the cytochrome system. These electrons are passed from one cytochrome to another until the reduced form of the enzyme, cytochrome oxidase is formed. In the process, $NADH_2$ is converted to NAD^+, and ATP is generated (43:27). It should be pointed out that the bulk of the energy of glucose is obtained from oxidation with 15 moles of ATP being formed from each mole of pyruvate oxidized (17:19).

Influence of Exercise upon Glucose and Glycogen

Glucose

Studies by Randle and Kipnis (30, 32, 36, 37) demonstrated that an anoxic condition promoted the transport of glucose across the muscle cell membrane. Goldstein (16), using eviscerated-nephrectomized rats and dogs which, at rest, were unable to utilize sugars, including isomers of glucose, indicated that muscular work increased the rate of glucose uptake by the tissues. Under such experimental conditions, muscular work promoted cellular entry of some of these sugars. This action of muscular work was independent of insulin as was exhibited in the depan-

THE KREBS CYCLE (TRICARBOXYLIC ACID CYCLE) (21:250)



Enzymes Involved

1 Pyruvic dehydrogenase
2 Condensing enzyme
3 Aconitase
4 Isocitric enzyme
5 α-Ketoglutarate dehydrogenase
6 Succinic thiokinase
7 Succinic dehydrogenase
8 Fumarase
9 Malic dehydrogenase

creatized preparations. Both insulin and a humoral product of contracting muscle could apparently activate a transfer mechanism for certain types of sugars across a cell surface barrier.

Non-working rats were compared with anaesthetized rats subjected to continuous intravenous infusions of C^{14}-glucose over a period of twelve hours with faradic stimulation of either one or both hind legs. The urinary excretion and the hepatic storage in isotopic glucose were suppressed by muscular work. This muscular work also caused a marked increase in the oxidation of isotopic glucose (28).

Blood samples collected during exercise and rest periods from a human subject given an intravenous injection of a trace dose of glucose-U-C^{14} indicated that stored muscle glycogen represented the immediate fuel for glycolysis; that is, there was an obligatory primary breakdown of glycogen before blood glucose could enter the muscle cells (38). Subsequently, there was an increased uptake of the blood glucose by working muscle, compensated in this experiment by an increased hepatic glucose output. The data were in accord with previous suggestions (16) that there is a mechanism whereby muscular work stimulates glucose uptake. The increased rate of decline in blood glucose specific activity, which occurred after work had begun, signified a rapid outpouring of hepatic glucose, and since the blood sugar level did not increase, the removal of glucose by the exercising muscles must likewise have correspondingly increased.

Young et al. (47) claimed that their study was the first demonstration of increased glucose production in man after prolonged exercise. An increased synthesis of glucose was demonstrated. During exercise

and in the steady state, the production rate was 206 mg/kg/hour which tended to maintain the blood glucose at a stable level while simultaneously providing fuel for oxidative metabolism.

Glycogen at Submaximal Exercise

Most of the research carried out with human subjects utilizing the muscle biopsy technique to examine the effects of exercise upon muscle glycogen have been conducted by Saltin, Hermansen, Hultman, and Bergstrom (2, 7, 8, 23, 24, 25, 39). The muscle biopsy technique has enabled researchers to measure muscle glycogen levels in man during various stages of exercise. Previously, cadavers, amputated limbs, or sacrificed animals were the main sources available in determining muscle glycogen levels (23:11).

Procter and Best (35), in a classical study of muscle glycogen, trained healthy dogs to run on three legs by tying one of the hind legs loosely, by means of a tape, to a strap about the animal's abdomen. They exercised the dogs for two to three weeks, and after sacrificing the animals, analyzed the rectus femoris muscle of both hind legs for comparison. It was suggested that there may be an optimum period of training for the accumulation of glycogen. Muscles trained longer than this optimum time retained their increased ability to perform work, but showed no extra increase in glycogen levels beyond that achieved from the optimum training period. A raised glycogen content of skeletal muscle did not appear to be an essential factor in the increased efficiency which resulted from training.

Grollman (20) trained rats in an exercise wheel for one month and

then exercised trained and untrained animals to exhaustion. The trained rats had higher muscle glycogen values at the start of the final exercise bout and lower values at the end of exercise. These findings indicated that the increased stores of glycogen resulting from training could be useful in extending muscular endurance upon exhaustive exercise.

Hultman and Bergstrom's work (8) supported by Saltin and Hermansen (39) illustrated that during exercise a continuous utilization of muscle glycogen takes place, which was however, confined to the exercising muscle group. The glycogen breakdown in the working muscle group did not occur at the same rate throughout, but the first 15 to 20 minutes' exercise produced the greatest fall in glycogen per time unit. After this period, which was also associated with the largest production of lactic acid, the local consumption of glycogen in working muscle was much lower and largely constant and then fell to an extremely low level during the last minutes of work. The above seemed to indicate that the glycogen breakdown curve was probably triphasic (8). If the work was continued to total exhaustion, no great decrease took place in the glycogen store during the last few minutes. Hultman (23:32) believed that, during this period, the energy production was derived from the following factors: a local increase in fat consumption in the glycogen-depleted muscle as well as some consumption of locally accumulated lactic acid; some local increase in the utilization of glucose supplied via the blood stream which resulted in a slight fall in blood sugar, but was compensated for by an increased release of glucose from the liver; and an increased activity in other muscle groups with a higher glycogen content, which may possibly have deputized for the glycogen-depleted

muscle.

It should be noted that Hultman (8) found that only working muscle groups utilized glycogen during the work period. Thus, no fall was noted in the glycogen content of resting muscle groups.

In conducting measurements of blood lactic acid concentration on cross-country skiers, Astrand (5) noted that the highest values for blood lactate concentration was found after the 10 km. race. The longer the distance of the competition the lower was the blood lactate concentration. This led to the conclusion that there was a different kind of fatigue limiting the physical performance depending on the duration of the severe exercise. Saltin (39) noted a similar pattern in the blood lactate level. There was a large initial increase in blood lactate level during the first 20 minutes, then as the exercise continued the lactic acid level dropped. The rate of glycogen reduction was greatest during the first 60 minutes, and then the reduction of glycogen proceeded at a slower rate from already low values. Higher lactate values were observed in untrained subjects when they were performing the same relative work loads as the trained subjects.

In their experiment on lactic acid removal in cats and dogs, Eggleton and Evans (14) concluded that, throughout recovery, the lactic acid content of the venous blood returning from the exercised muscles remained higher than that of the arterial blood. However, the lactic acid content of the returning venous blood from unexercised muscles remained lower.

Both Hultman (25) and Saltin (39) had similar observations regarding muscle glycogen levels during and after exercise. After controlling

the subjects' diets and exercise programs several observations were made. On a mixed diet (carbohydrate, fat, and protein) the glycogen content was in the range of 1.5 to 2.5 g/100 g. wet muscle tissue, and the work time was 85 to 145 minutes. After the fat and protein diet the muscle glycogen was only 0.3 to 1.3 g/100 g. wet muscle tissue. The decrease in work time was of the same relative magnitude. On the carbohydrate diet the muscles were able to build up a very high glycogen content of over 3 g/100 g. wet muscle tissue in some cases. The work time was again closely related to the muscle glycogen content. A high carbohydrate diet preceded by a fat and protein diet produced the highest glycogen levels. The low glycogen level created by the fat and protein diet seemed to be overcompensated for by the same mechanism when the carbohydrate replaced the fat and protein diet. In these studies, both Hultman (24:44) and Saltin (39) agreed that the ability to perform very heavy prolonged exercise was directly related to the glycogen stores of the body and in particular of the muscle.

Ahlborg (2) infused glucose into 2 subjects during exercise. The lowering of glycogen was of the same order as when the subjects exercised without administration of glucose, despite the fact that the glucose content in the arterial blood was greatly elevated. This suggested that the glycogen catabolism during exercise takes place at almost the same rate irrespective of the quantity of glucose delivered to the muscles; that is, there appeared to be an obligatory breakdown of muscle glycogen. It also suggested that the muscle glycogen content is an important limiting factor in the ability to exercise even when the blood

glucose concentration is high because of the inability to get sufficient glucose across the muscle cell membranes.

CHAPTER III

METHODS AND PROCEDURES

A group of 29 male subjects ranging in age from 17 to 28 were divided into three groups; sedentary, semi-active and active. The active group underwent training in preparation for competition in university wrestling or judo. The wrestlers' training program lasted 4 months and included running stairs, weight training and hour-long practices 4 or 5 times per week as well as regular competitions. The judo training extended over the same seasons and included hour-long practices 3 to 5 times weekly as well as supplementary running. The semi-active group continued to exercise regularly in activities such as squash, handball, swimming, running, weight training and other intramural sports. However, they did not undergo a concentrated training program. The sedentary group remained relatively inactive.

At the initial stages of the experiment and prior to the training program, the active group was tested for maximal work capacity on a bicycle ergometer. Each subject, with chest electrodes in place, mounted a Monark bicycle ergometer, and the heart rate was recorded by means of a Sanborn 100 viso-electrocardiogram while the subject pedalled at 50 r.p.m. The work load was set anywhere from 1.5 to 3.5 kiloponds depending upon the tester's estimate of the subjects's fitness. The heart rate was recorded at the end of every minute for 6 minutes, and the rate at the end of the sixth minute was regarded as the steady state. Each subject was tested at least twice within a two week period, but if the 2 steady state heart rates differed by more than ± 4 b.p.m. the subject

was required to repeat the test until 2 values were found within the acceptable range for predicting a MVO_2 from the Astrand-Rhyming Nomogram (6). The work load of 50% MVO_2 was then calculated from the nomogram.

From October, 1968 to February, 1969 all subjects underwent both the submaximal and maximal tests during which muscle samples were taken. The active group was tested in October and November prior to their competitive season and then retested 3 or 4 months later near the end of their training programs. Prior to the muscle sampling tests the subjects or their guardians signed a consent form agreeing to participate in the study.

Muscle samples were taken at various times before, during and after the submaximal and maximal tests from a working muscle, the vastus lateralis. The biopsy needle consists of a sharp-edged aluminum cylinder fitting lightly into a pointed hollow outer cannula (3.5 to 5.0 mm. in diameter) with a small opening near the tip (7) (see diagram in appendix B).

A few minutes after a local anaesthetic xylocaine hydrochloride was injected, an incision of the skin approximately 6 to 10 mm. was cut with a scalpel. The biopsy needle was then advanced 4 to 5 cm. into the muscle. The cylinder was pulled back a few centimetres while the cannula remained in place and then was pushed in again, cutting off a small piece of muscle (approximately 30 to 50 mg.) which protruded into the opening of the needle. The muscle sample was then transferred to the weighing pan, and all visible fat, connective tissue, and blood were removed using a probe, forceps, and gauze. The sample was then weighed on a Roller-

Smith Precision Balance and transferred with forceps to the bottom of a capped test tube. Immediately the sample in the test tube was frozen in dry ice and 95% ethyl alcohol and left in the mixture until the end of the testing session. The pan, with traces of blood still remaining, was again weighed, and the weight of the blood subtracted from the previous weight to give the weight of the sample in the test tube. After the testing session all of the frozen muscle samples were stored frozen until analyzed for glycogen content.

No effort was made to control the subjects' diets except that they were asked to abstain from alcohol for 24 hours prior to testing. The testing sessions were from 4:30 to 7:00 P.M. on week days and 9:30 to 12:00 A.M. on Saturdays. With a few exceptions, the subjects participated in the submaximal test first.

Initially 2 biopsies were taken from the resting subject's right thigh. The subject then mounted a bicycle ergometer and commenced pedalling at 50 r.p.m. at the work load estimated to require 50% MVO_2. After every 20 minutes of exercise the subject stopped momentarily so that a biopsy could be taken. At the end of every half-hour of pedalling the work load was increased by one-third of the previous level. When the subject could no longer pedal at the required work load and rate the exercise was stopped, and a sample was taken. Ten minutes after the cessation of exercise a further biopsy was removed.

Within a period of no less than a week after the submaximal test each subject was required to undergo the maximal test. First a sample was taken from the vastus lateralis of the reclining individual. The subject then mounted the bicycle ergometer and commenced pedalling at

the same rate and work load as at the initial stage of the submaximal test. At this testing session the work load was raised each minute by one-third of the previous level. Immediately at the end of exercise a muscle sample was taken. After 10 minutes of rest, another sample was removed from the subject.

At the end of the training program in February and March, 1969, all physiological measurements were taken from the active group in a manner similar to the pre-training tests. This involved a repeat of preliminary tests to estimate the work load required for 50% MVO_2 as well as the submaximal and maximal tests during which muscle biopsies were taken.

The muscle samples were analyzed for glycogen content at the Surgical Medical Research Institute Laboratory at the University of Alberta. The frozen muscle tissue in the capped test tube was first digested with .5 ml. of 30% KOH saturated with Na_2SO_4. To hasten the digestion of the muscle tissue inside the test tube, the tube was heated in a boiling water bath for 20 to 30 minutes or until the tissue was completely digested. The sample was then cooled in an ice bath for 10 minutes (38, 39).

To precipitate the glycogen from the solution, .8 ml. of 95% ethanol (approximately 1.2 volumes) were added (18). At this stage the solution was left to stand overnight and the following morning put in an ice-bath for one-half hour. After centrifugation at 840 x g. for 20 to 30 minutes the supernatant was carefully aspirated with a Pasteur pipette. To the precipitate in the test tube were added 3 cc. of distilled water to dissolve the glycogen precipitate. The solution was then agitated to ensure that all the glycogen had dissolved. In a 150 x 20 mm. test tube

an appropriate aliquot (.2 ml.) of the glycogen solution was diluted to 1 ml. with distilled water (.8 ml.). Triplicate samples were prepared to minimize errors resulting from accidental contamination with cellulose lint. One ml. of 5% phenol solution was added to each of the above as well as to a reference sample of 1 ml. of distilled water. To each test tube 5 ml. of 96% sulphuric acid were added rapidly, the stream of acid being directed against the liquid surface rather than against the side of the test tube in order to obtain good mixing (13). The tubes were allowed to stand for 10 minutes, then were shaken and placed for 10 to 20 minutes in a water bath at 25° to 30°C before colourimetric readings were taken in the following manner.

The absorbance for each of the triplicate samples was read at a wavelength of 490 mμ. on a Beckman model DU-2 ultra-violet spectrophotometer. The glycogen content of the sample was then read from the standard curve which had previously been constructed for glycogen in the following way: triplicate samples of standard glycogen solutions containing from 5 μg. to 100 μg. glycogen were subjected to the same phenol and sulphuric acid reactions as were the muscle samples, and the average readings of absorbance were plotted against the amount of glycogen used, to produce a standard curve. In order to verify that the colour product from the phenol-sulphuric acid colourimetric method was stable some standard glycogen solutions were subjected to the same procedure and absorbance readings were taken from time to time over a period of 2 hours.

It should be noted that an average of 15 to 20 samples were analyzed per day. This included a standard sample run as a daily check on the standard curve.

Six one-way analyses of variance (15:284) were used to compare the means of the initial, final, and final resting glycogen levels for each group (4 groups) on both the maximal and submaximal tests. Eight additional analyses of variance were run to test for significant differences among the means of the initial, final, and final resting glycogen levels within each group for both the maximal and submaximal tests. As well, an analysis of variance was used to compare the pre-training and post-training MVO_2 for the active group. Tukey's w-procedure (40:109) was used to establish significant differences between means. Curves were constructed to demonstrate glycogen depletion with maximal and submaximal exercise.

CHAPTER IV

RESULTS

Figure I shows the standard curve for glycogen using the phenol-sulphuric acid method. The absorbance for the varying concentrations of glycogen were read at wave length 490 mμ.

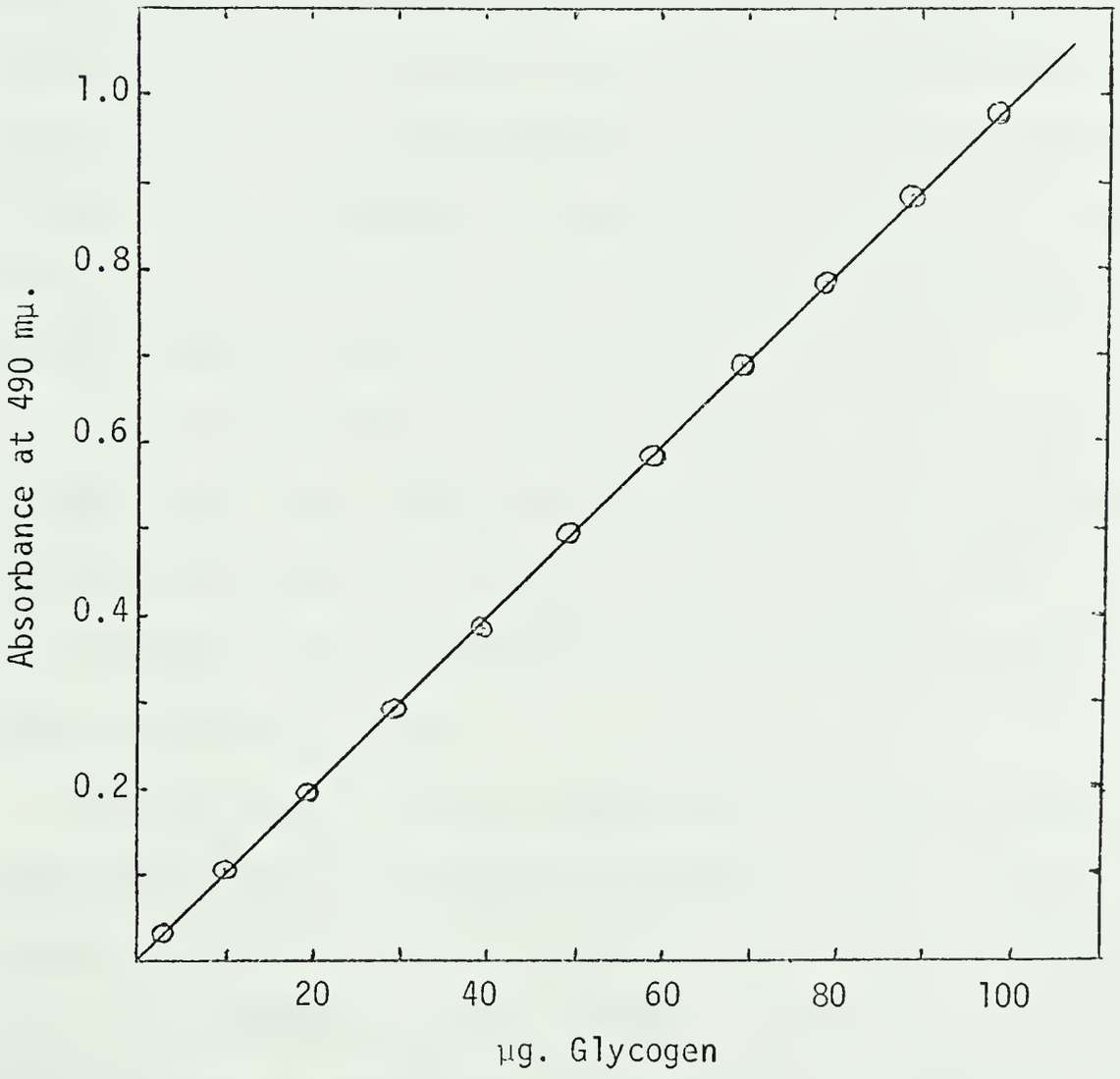


FIGURE I STANDARD CURVE FOR GLYCOGEN USING THE PHENOL-SULPHURIC ACID COLOURIMETRIC METHOD

Table 1 indicates the mean glycogen levels (grams of glycogen/100 grams wet muscle) and the standard error of the mean (S.E.M.) for the samples taken prior to exercise, at the point of fatigue, and after a 10 minute rest for a submaximal test. The individual analyses of variance for significant differences between means ($p < 0.05$) may be found in the appendix. In utilizing Tukey's w the values were used for testing significant differences ($p < 0.05$) within individual groups and also to test for significant differences between groups for initial, fatigue, and resting values. Any two means differing by more than the value of Tukey's w were considered to be significantly different at the 0.05 level. Although 29 subjects participated in the study, some samples were rejected due to faulty biochemical determination and excessive connective and lipid tissue within the muscle.

In all groups there was a significant ($p < 0.05$) change in muscle glycogen level from initial to fatigue; however, no significant difference existed between the fatigue and final resting values. A significant difference still remained between the initial glycogen stores and after 10 minutes of rest.

For the initial values a significant difference existed only between the active post-training and sedentary groups. However, a trend showing a decrease in initial glycogen levels from the active post-training to the sedentary group was evident. Generally, for the fatigue and resting values no significance was found and no trends were noted.

Table 2 indicates the mean glycogen levels (grams of glycogen/100 grams wet muscle) and the standard error of the mean (S.E.M.) for the samples taken prior to exercise, at the point of fatigue, and after a

TABLE 1 GROUP MEAN MUSCLE GLYCOGEN LEVELS FOR THE SUBMAXIMAL TEST

GROUP	INITIAL	FATIGUE	REST 10 MIN.	TUKEY'S w($p < 0.05$)[c]
SEDENTARY (5)[b]	1.03 ± 0.08[a]	0.59 ± 0.13	0.76 ± 0.14	0.30
SEMI-ACTIVE (11)	1.21 ± 0.10	0.65 ± 0.07	0.62 ± 0.08	0.24
ACTIVE PRE-TRAINING (10)	1.22 ± 0.10	0.52 ± 0.10	0.69 ± 0.12	0.30
ACTIVE POST-TRAINING (11)	1.33 ± 0.15	0.73 ± 0.12	0.62 ± 0.09	0.30
TUKEY'S w($p < 0.05$)[c]	0.22	0.20	0.20	

a Values are means ± S.E.M. in grams of glycogen/100 grams wet muscle.

b Numbers in parentheses are subjects per group.

c Means differing by more than Tukey's w indicate significant changes ($p < 0.05$).

TABLE 2 GROUP MEAN MUSCLE GLYCOGEN LEVELS FOR THE MAXIMAL TEST

GROUP	INITIAL	FATIGUE	REST 10 MIN.	TUKEY'S $w(p < 0.05)$[c]
SEDENTARY (4)[b]	0.86 ± 0.07[a]	0.59 ± 0.11	0.73 ± 0.16	0.28
SEMI-ACTIVE (11)	1.24 ± 0.11	1.09 ± 0.13	0.94 ± 0.07	0.22
ACTIVE PRE-TRAINING (10)	1.18 ± 0.10	1.10 ± 0.09	0.97 ± 0.10	0.20
ACTIVE POST-TRAINING (11)	1.61 ± 0.14	1.23 ± 0.16	1.21 ± 0.13	0.30
TUKEY'S $w(p < 0.05)$[c]	0.28	0.28	0.23	

a Values are means ± S.E.M. in grams of glycogen/100 grams wet muscle.

b Numbers in parentheses are subjects per group.

c Means differing by more than Tukey's w indicate significant changes ($p < 0.05$).

10 minute rest for a maximal test. Tukey's w may be utilized in the same manner as for Table 1, and the individual analyses of variance can be found in the appendix.

On the maximal test only the sedentary and active post-training groups experienced a significant decrease ($p < 0.05$) in muscle glycogen level from the start of exercise until fatigue. Generally a decrease in glycogen from the fatigue to the final resting samples existed, but none of these differences was significant. With the exception of the sedentary group a significant difference between the initial and final resting glycogen levels was observed.

A large difference between the active post-training and sedentary groups for the initial values was seen. This large significant difference was evident for both the fatigue and final resting glycogen levels as well. The initial, fatigue, and final resting values were similar for the semi-active and active pre-training groups. Both of these groups generally differed significantly from the sedentary and active post-training groups with glycogen values lying between the two extremes.

Figure II contains curves demonstrating the decrease in the glycogen levels for each group on the submaximal test. The number of samples taken from a subject during the submaximal test varied from 4 to 8 depending upon the length of the exercise time. All the initial, fatigue, and resting samples respectively were considered to be biologically equivalent, regardless of exercise time.

The X-axis is in units of percentage of total work time. For each group 8 points were plotted. These points were the mean glycogen levels for samples falling at or between the following percentages of total

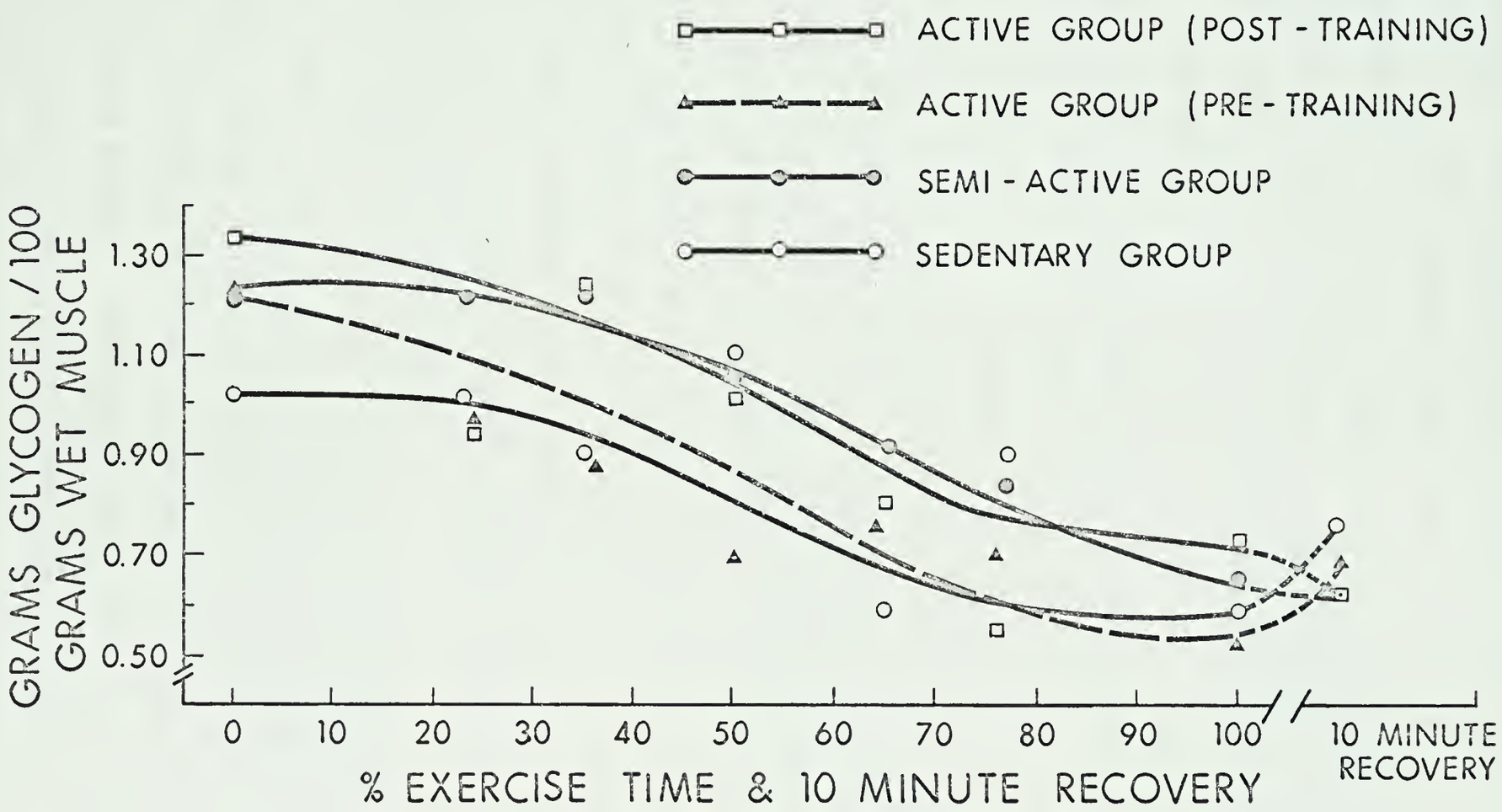


FIGURE II GLYCOGEN LEVELS OF ALL GROUPS FOR SUBMAXIMAL WORK LOADS

work time: 0%, 16-2/3% to 25%, 33-1/3% to 40%, 50%, 60% to 66-2/3%, 75% to 83-1/3%, 100%, and the final resting value. In order to establish a point where a percentage range was given, the values falling within a particular area were weighted as to sample number. After the points were plotted curves of best fit were drawn by eye for each group.

Although the initial values varied for the groups, the curves were generally of the same shape, showing a steady decline in glycogen content with a plateauing effect near the termination of exercise and during the rest period. Throughout, the glycogen levels for the active-trained group were generally the highest while the values for the sedentary group were the lowest. The glycogen levels for the remaining 2 groups fell between the sedentary and active-trained values.

Figure III demonstrates the curves of the glycogen levels for each group on the maximal test. Although the working times varied between 3 and 12 minutes, all the initial, fatigue, and resting samples respectively were considered to be biologically equivalent. Each point that was plotted represented the mean glycogen level of all corresponding samples for a particular group.

The curves illustrated a small but steady decline in the glycogen levels until fatigue. Both the sedentary and active-trained groups exhibited a slight recovery in glycogen level during the 10 minute rest while a negligible decrease continued in the semi-active and active pre-training groups. Generally, however, the curves are of the same shape although the initial glycogen values do differ.

Both the curves for the submaximal and maximal tests exhibited a progressive decrease in muscle glycogen. However, the submaximal fatigue

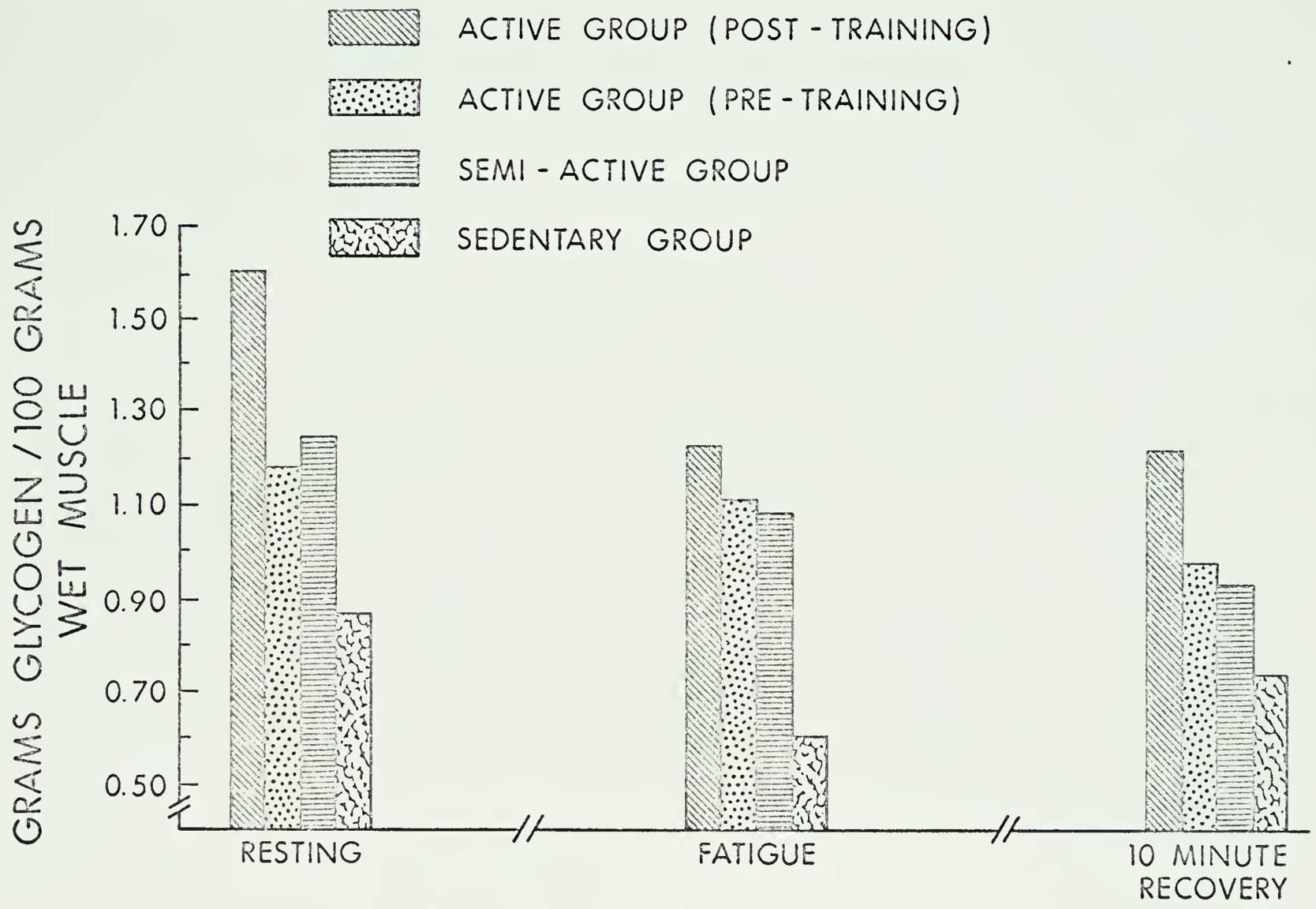


FIGURE III GLYCOGEN LEVELS OF ALL GROUPS FOR MAXIMAL WORK LOADS

and recovery levels were considerably lower than for the maximal test.

CHAPTER V

DISCUSSION OF RESULTS

Three studies (13, 18, 45) were used to construct a technique for glycogen analysis. Since glycogen is stable in hot alkali the muscle sample was hydrolysed with 30% KOH in boiling water to form an homogeneous fluid (18). It was found that the glycogen is best precipitated from the alkaline hydrolysate by using 1.1 to 1.2 volumes of 95% ethanol (18). Higher alcohol concentrations prolonged glycogen separation time and rendered it impossible to obtain clear mother liquor either by filtration or by centrifugation. No such difficulty was encountered when the alcohol content of the mother liquor was kept below 60%. Dextrins were partially carried down at 65% and completely precipitated at 75% alcohol concentrations (18). By decreasing the alcohol concentration to 50%, it became possible to remove from the mixture glycogen alone (18). Since the glycogen was present in small amounts, saturated sodium sulphate solution was used as a coprecipitate for fast quantitative precipitation of glycogen (45). Heating after addition of ethanol to flocculate glycogen is unnecessary in the presence of sodium sulphate (45).

The anthrone colourimetric method was not used because the anthrone reagent is expensive and the solution in sulphuric acid was found to be unstable (13). The phenol-sulphuric acid technique was used since it can be utilized for the quantitative colourimetric microdetermination

of sugars and their methyl derivatives, oligosaccharides and polysaccharides. The method is simple, rapid, and sensitive. It also gives reproducible results. The colour produced was stable and possessed a definite absorption peak at 490 mμ. The sulphuric acid was added rapidly directly against the surface of the phenol-glycogen solution to give a high maximum temperature and to increase the sensitivity of the reagent (13). In the presence of sulphuric acid the glycogen is broken down to glucose which is further dehydrated to form 5-hydroxymethylfurfural. The 5-hydroxymethylfurfural is condensed with phenol to give the yellow-orange colour which is then measured.

Carbohydrates have been shown to be one of the main sources of energy during exercise (23:48). Findings have suggested that stored muscle glycogen represents the immediate fuel for glycolysis and respiration of working muscle (38). During prolonged exercise glycogen stores become depleted and reach minimum levels near fatigue (8, 39), indicating that the breakdown of glycogen was one of the critical factors for energy supply in the working muscle and that the speed of net breakdown of muscle glycogen affects the working time (3).

Hultman (23) noted higher initial muscle glycogen levels than were observed in this experiment. The mean pre-exercise value was 1.39 grams glycogen/100 grams muscle. In this study only the active post-training group experienced a glycogen level exceeding 1.39 grams glycogen/100 grams muscle (the value was 1.61 grams glycogen/100 grams muscle). Some of Hultman's trained cross country skiers had glycogen values over 2.5 grams/100 grams muscle. However, these athletes were fully trained, had performed hard prolonged exercise, and had eaten high carbohydrate

diets on the days before the test, a combination which proved to be effective in raising the muscle glycogen content (23:20). In fact, under the previous conditions Hultman noted glycogen levels as high as 4 to 5 grams/100 grams muscle in some of the highly trained cross country skiers (23:23). In contrast, subjects used in this study varied from sedentary individuals to trained wrestlers and judoka, but none of the trained group were as highly conditioned as cross-country skiers. The very nature of the three sports is different with wrestling and judo requiring short bursts of effort as opposed to muscular endurance in cross-country skiing.

During submaximal exercise a decrease in muscle glycogen has been found (2, 3, 8, 25, 35, 39). Some researchers noted mean glycogen levels as low as 0.27 grams glycogen/100 grams muscle at the point of fatigue. In contrast, during the submaximal test in this study the lowest mean glycogen level observed was 0.52 grams glycogen/100 grams muscle. The continuation of work beyond the time when a minimum glycogen level was reached has been attributed to a local increase in fat consumption in the glycogen-depleted muscle. Additional factors were: a local increase in the utilization of glucose supplied via the blood stream, an increased activity in other muscle groups with a higher glycogen content, and a consumption of locally accumulated lactic acid (23:32).

The limiting factor in muscular work is the availability of energy-rich phosphate in the muscle (26). Muscle glycogen is of vital importance for the resynthesis of phosphocreatine and ATP (3). However, perhaps the depletion of glycogen is not the limiting factor in fatigue.

Possibly the limiting factor lies not in actual muscle glycogen stores but in the presence of enzymes essential to change glycogen to glucose-1-P. The present consensus appears to be that phosphorylase 'b' rather than 'a' predominates in resting muscle (41). During exercise phosphorylase 'b' is converted to the active form phosphorylase 'a' by the muscle enzyme phosphorylase 'b' kinase (41). The activated phosphorylase 'a' in turn aids in breaking down the glycogen to glucose-1-phosphate. Conceivably the amount of phosphorylase 'b' or phosphorylase 'b' kinase present in the muscle could be the deciding factor in the amount of glycogen that is made available for the resynthesis of phosphocreatine and ATP. An investigation is presently being conducted to examine muscle phosphorylase levels during exercise.

Since the work load was increased by one-third every half hour, near the point of fatigue at the final work load the subject was working primarily anaerobically at from approximately 85% to 95% of his MVO_2. In fact, during this period the work could actually be considered maximal instead of submaximal. Therefore, it is possible that circulatory factors (1, 42) predominated near the end of exercise. That is, since the subject was working anaerobically, excess lactic acid accumulated in the muscle tissue and energy-rich phosphates were not resynthesized rapidly enough due to an insufficient oxygen supply. This type of increased work load could account for the higher fatigue glycogen level existing in the present experiment.

After 10 minutes of rest no significant changes in muscle glycogen levels from the point of fatigue were noted. Ten minutes was obviously not sufficient time for the resynthesis of muscle glycogen to occur.

In fact, Ahlborg (2) observed that there was no significant increase in glycogen even after a one-hour rest.

No literature illustrating the effects of a training program upon the muscle glycogen levels of humans appears to be available. However, dogs (35) and rats (20) which exercised for 2 or more weeks showed a considerable increase in the glycogen content of certain trained muscles. These results indicated that there may be an optimum period of training for the accumulation of glycogen. Training also enabled the animals to utilize more glycogen. The trained group in the exhausted state demonstrated a glycogen concentration of 38.1% less than the untrained (20).

In the present study the active post-training group had a significantly higher initial glycogen level (1.33 grams glycogen/100 grams muscle) ($p < 0.05$). Although the active group experienced a rise in initial glycogen level from pre-training to post-training the difference was not significant. Prior to the training program for the active group, the active and semi-active groups were of comparable fitness level, and their glycogen levels before, at the end of, and after exercise were also similar. In general, the glycogen levels of the groups at the point of fatigue remained in the same relative rank as the initial values. There did not appear to be an increased utilization of muscle glycogen for the active-trained group. In fact, the fatigue level was 0.73 grams glycogen/100 grams muscle, the highest of all four groups. Therefore, from this study, it does not appear that trained humans deplete glycogen stores to lower values than untrained subjects under submaximal exercise conditions.

Glycogen synthetase exists in two enzymatically interconvertible

forms: synthetase I, active in the absence of glucose-6-phosphate, and synthetase D with little or no activity unless glucose-6-phosphate is present. In living muscle the relative distribution of these two forms of glycogen synthetase is related to the tissue concentration of glycogen in such a way that the synthetase I increases when the concentration of glycogen falls. Glycogen possibly controls its own synthesis through a feedback system in which an as yet undefined mechanism affects the interconversion of glycogen synthetase I$\rightleftharpoons$D in such a manner as to favour glycogen synthesis when tissue concentrations are low, and to slow synthesis when tissue concentrations are high (11). Trained guinea pigs had greater glycogen synthetase activity than untrained (29). Therefore, in this study perhaps the higher initial muscle glycogen levels in the trained active group could be explained by a similar increased glycogen synthetase activity. No effort was made to control the subjects' diets other than requesting abstinence from alcohol for 24 hours before testing. Alcohol consumption prior to testing could have created an additional source of energy for exercise since ethanol is broken down into acetyl CoA which enters the Krebs cycle and results in the production of ATP (34). The increased muscle glycogen values after training could partially be accounted for by an unreported high carbohydrate diet near the end of the wrestling and judo seasons, especially in the case of the wrestlers who dieted frequently to make a weight division.

In other studies (8, 39) when individuals exercised submaximally the curve showed an initial rapid decrease in muscle glycogen, followed by a constant fall, and finally, during the last minutes of work, a slower disappearance. The types of work varied from intermittent exercise periods

(usually 15 to 20 minutes of work followed by a corresponding rest) to long 3 hour sessions. Generally, the work load remained constant throughout the total work period. In this study, the glycogen levels decreased slowly at the beginning followed by a more rapid depletion from 25% to 75% of total exercise time. From 75% of the total time till the end of the rest period the glycogen levels appeared to plateau near the minimum glycogen value attained. Curves such as these should be expected since each subject started working at 50% MVO_2 and worked progressively harder as the work load was increased by one-third every half hour. During the last 25% of exercise time when the work loads were highest the subjects did not experience a large decrease in muscle glycogen. Possibly, as previously mentioned, their working muscles were unable to utilize glycogen below this level due to the absence of an enzyme or enzymes necessary for the breakdown of glycogen. Since the work load was high in the last 25% of exercise it is also quite possible that circulatory factors (1, 42) caused fatigue before the glycogen could be completely depleted. Since the subject was working anaerobically, excess lactic acid could have accumulated in the muscle tissue and ATP, the energy source, was not resynthesized rapidly enough due to an insufficient oxygen supply.

No studies were available to examine human muscle glycogen levels in connection with a short maximal work effort. In this experiment the maximal exercise usually lasted approximately 4 to 7 minutes before fatigue. The initial load required the subject to work at 50% MVO_2, and the work load was raised by one-third of the previous level every minute. As noted for the submaximal test there was a significant

difference ($p < 0.05$) between the initial values for the active-trained (1.61 grams glycogen/100 grams muscle) and sedentary groups (0.86 grams glycogen/100 grams muscle). Significant differences ($p < 0.05$) also existed between the active-trained group and the semi-active and active pre-training groups. Also, as with the submaximal test, the semi-active and active pre-training groups exhibited almost identical glycogen levels throughout the entire maximal test since they possessed comparable fitness levels.

At the point of fatigue only the mean glycogen content of the active-trained group differed significantly ($p < 0.05$) from the initial level. Since very little of the glycogen store was utilized this would seem to indicate that during maximal work, glycogen certainly is not the limiting factor at the point of fatigue. As previously mentioned, circulatory factors (1, 42) resulting in the accumulation of lactic acid and insufficient oxygen for the resynthesis of energy-rich phosphates likely predominate.

A plateauing of the glycogen level after cessation of exercise was observed. Obviously 10 minutes was not sufficient time for resynthesis of glycogen to occur. The sedentary group did exhibit a near significant increase in glycogen during the 10 minute rest, but this rise probably could be attributed to experimental error since only 4 valid glycogen values were available to plot this point. In agreement with the submaximal test the trained group did not exhibit any increased ability to utilize glycogen to lower levels at the point of fatigue. The curves were similarly shaped and remained somewhat parallel throughout.

In this study there appeared to be a training effect upon the initial glycogen levels for the active group, possibly due to increased glycogen synthetase activity. For both the submaximal and maximal tests the active post-training group had the highest glycogen value followed by the active pre-training and semi-active groups and then by the sedentary group. Generally the pre-exercise glycogen levels were lower than the values obtained in Scandinavia perhaps because of the lower fitness level of the Canadian subjects used. Higher levels of training did not appear to enhance the subject's ability to utilize more glycogen for either the submaximal or maximal work. In addition, resynthesis of glycogen did not occur after 10 minutes rest for either type of work load.

For the submaximal work loads the curves for all groups were similarly shaped. Such was also the case for the maximal work loads. However, the curves for the two types of tests differed with the submaximal curves showing a lower glycogen level than the maximal curves. Evidently glycogen does not govern fatigue under the working conditions used in this experiment. Possibly the highly-trained Scandinavians possessed some additional adaptation acquired from more extensive training to allow them to deplete their glycogen stores to much lower levels than observed in this study.

This study is the first known to compare the glycogen levels of working muscle in the same human subjects under submaximal and maximal exercise conditions and to examine the effects of training upon these levels.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The purpose of this thesis was to examine the glycogen levels of working skeletal muscle and the adaptations of the muscle in a trained and untrained state to maximal and submaximal work loads.

Twenty-nine subjects from the University of Alberta were divided into sedentary, semi-active, and active groups. At the initial stages of the experiment all 3 groups were tested for MVO_2 on a Monark bicycle ergometer. The work loads for 50% MVO_2 were then calculated from the Astrand-Rhyming nomogram. All subjects underwent both the submaximal and maximal tests during which muscle biopsies were taken from a working muscle, the vastus lateralis. For both tests the subjects started working at 50% MVO_2. On the submaximal test the work load was raised by one-third of the previous value every 30 minutes. Biopsies were taken before exercise, after every 20 minutes of work, at the point of fatigue, and after a 10 minute rest. For the maximal test the work load was raised by one-third of the previous value every minute of exercise and 3 samples were taken: before exercise, at fatigue, and after a 10 minute rest. The active group, consisting of wrestlers and judoka, trained for 3 or 4 months in their respective sports, and then all physiological measures were taken again. At the end of all testing the muscle samples which had previously been frozen and stored were analysed for glycogen content using the phenol-sulphuric acid colourimetric technique.

For the submaximal work loads significant changes ($p < 0.05$) from initial to fatigue glycogen levels were observed. However, no significant differences were noted between the fatigue and final-resting values. Concerning the initial values, a significant difference ($p < 0.05$) existed only between the sedentary and active post-training groups, but a trend was evident from a high level in the active post-training group to a low in the sedentary group.

For the maximal test only the active post-training and sedentary groups had a significant difference ($p < 0.05$) in glycogen levels from initial to fatigue values. The other groups showed a small decrease. A large difference was observed between the initial glycogen levels of the sedentary and active post-training groups. The initial values for the other 2 groups were identical and differed significantly from both the minimal values of the sedentary group and the maximal values of the post-training group. In agreement with the submaximal test, generally no significant differences were seen between the fatigue and final-resting values.

All 4 curves for the submaximal and maximal work loads respectively were similarly shaped. Glycogen values at the point of fatigue were lower for the submaximal than for the maximal but still not as low as previously reported in the literature.

Conclusions

Training apparently does not enhance the ability to deplete muscle glycogen stores to lower levels during either submaximal or maximal

work. Depletion of muscle glycogen is not the critical factor causing fatigue when progressively increasing work loads are used for either submaximal or maximal work. This study has not substantiated early work demonstrating increased glycogen stores with training.

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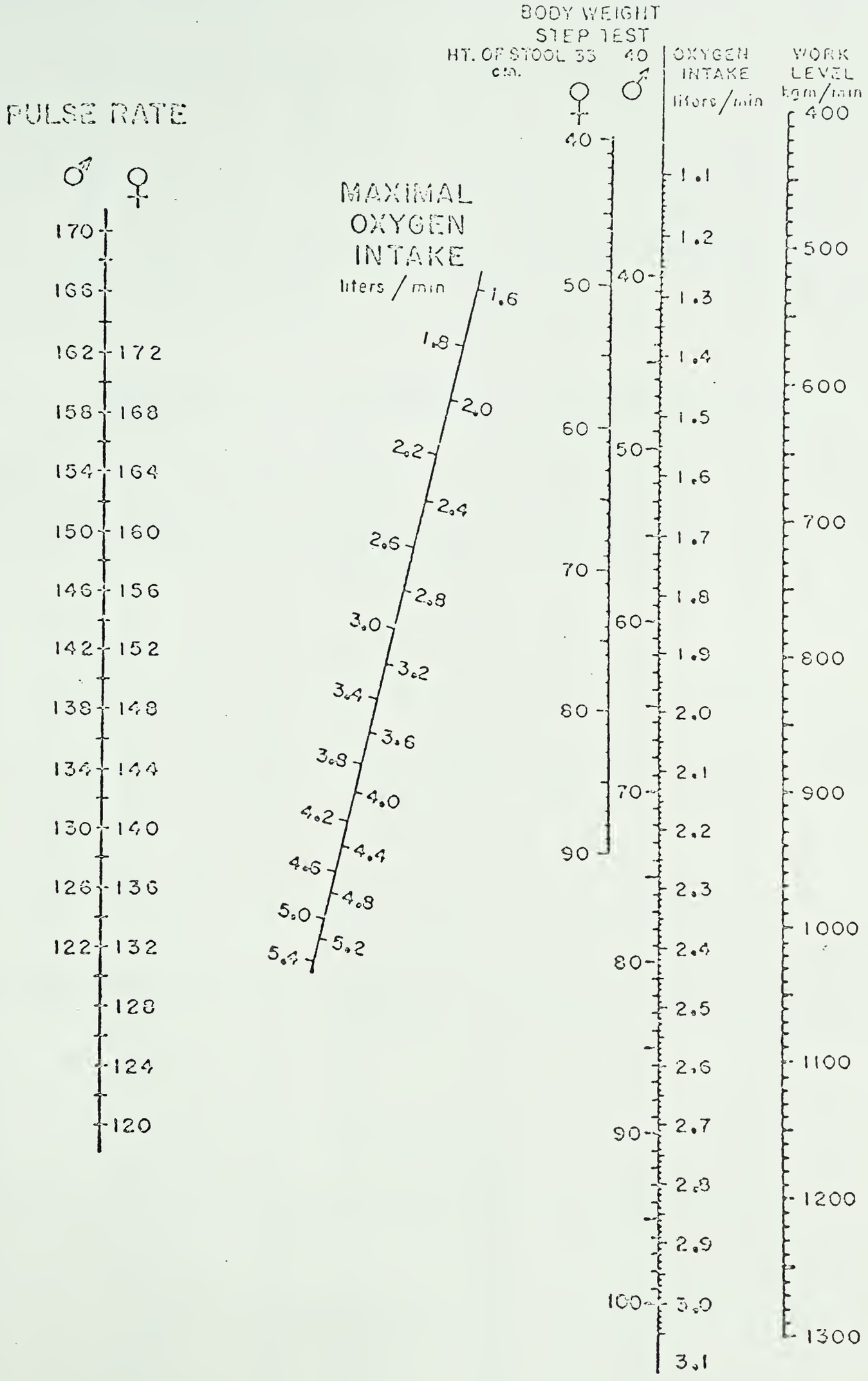
APPENDIX A



1. ASTRAND-RHYMING NOMOGRAM (6)

NAME	AGE	HEIGHT/WEIGHT	CLASSIFICATION

Astrand Rhyming Test for MVO_2

Stage	1	2	3	4	5	6	Steady State
ST 1							
ST 2							
ST 3							

VO_2 =	V.L =	70% =	V.L. =	Average S.S =	

MUSCLE BIOPSY SAMPLING

Date				Muscle Samples										
Time of Day	Diet Previous Day + Week	Activity During Week	Length of Ex.	Before	20	40	60	80	100	120	140	160	Rest 10 Min	Comments
				Before	During		After		10 Min					

3. TABLE OF AGE, WEIGHT, HEIGHT, STEADY STATE HEART RATE AND CORRESPONDING WORK LOAD, MVO_2, INITIAL LOAD, SUBMAXIMAL AND MAXIMAL WORK TIMES

Subj.	Age	Weight (lbs.)	Height (ins.)	Steady State H.R.	Work Load	MVO_2 (ℓ./min.)	Initial Load	Submax. Work time (min.)	Max. Work time (sec.)
					SEDENTARY				
W.G.	22	160	70	161	750	2.5	450	95	337
D.M.	21	150	68	144	600	2.5	450	78	330
B.L.	20	140	64	136	600	2.8	550	70	280
G.M.	20	160	68	156	600	2.2	400	120	432
R.S.	21	160	72	127	750	3.6	700	65	240
W.H.	24	195	68	136	600	2.8	550	55	277
J.S.	22	190	71	128	750	3.6	720	60	315
					SEMI-ACTIVE				
G.W.	27	155	71	160	900	2.8	550	87	330
R.P.	18	142	70	158	750	2.5	450	123	305
S.D.	21	161	70	130	750	3.6	720	80	396
K.M.	23	171	69	145	900	3.4	700	93	700
B.Y.	25	183	70	148	1050	3.8	780	65	288
J.G.	27	166	71	130	900	4.1	870	58	250
B.P.	22	135	66	163	750	2.4	450	85	341
G.H.	26	215	77	148	900	3.3	630	75	300
J.R.	24	165	69	148	750	2.8	550	80	341
M.B.	26	164	71	143	900	3.5	750	49	245
A.T.	28	195	72	138	900	3.7	750	50	260

..... continued

Table 3 continued ...

Subj.	Age	Weight (lbs.)	Height (ins.)	Steady State H.R.	Work Load	MVO_2 (ℓ./min.)	Initial Load	Submax. Work time (min.)	Max. Work time (sec.)
				ACTIVE PRE-TRAINING					
R.L.	23	186	72	139	1200	4.8	1050	78	403
T.T.	22	165	69	138	750	3.2	600	80	320
W.R.	21	160	73	143	750	3.0	570	68	310
A.M.	20	150	67	131	750	3.5	750	80	313
D.C.	19	153	71	122	750	4.0	840	56	264
L.A.	18	154	70	147	750	2.8	550	90	312
B.S.	24	170	69	131	900	4.0	840	68	268
K.S.	17	135	66	155	900	3.0	570	70	311
H.R.	24	162	67	122	750	4.0	840	60	236
B.J.	21	145	65	152	750	2.7	510	76	300
D.J.	22	137	69	145	750	2.9	540	80	300
				ACTIVE POST-TRAINING					
R.L.	23	186	72	126	1200	5.8	1170	40	315
T.T.	22	160	69	138	750	3.2	600	95	320
W.R.	21	150	73	141	750	3.0	570	70	300
A.M.	20	150	67	130	750	3.6	750	88	355
D.C.	19	153	71	126	900	4.4	930	54	286
L.A.	18	154	70	128	750	3.7	750	60	260
B.S.	24	170	69	122	900	4.7	960	60	230
K.S.	18	129	66	129	900	4.2	870	33	210
H.R.	24	160	67	132	750	3.5	700	65	235
B.J.	21	142	65	143	750	3.0	570	80	390
D.J.	22	133	69	132	750	3.5	690	80	336

APPENDIX B

UNIVERSITY OF ALBERTA
FITNESS RESEARCH UNIT and
FACULTY OF PHYSICAL EDUCATION

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

SUBJECT . . . . . DATE . . . . . TIME A.M. / P.M.

1. I agree to participate in an investigation and in relation to this hereby authorize Drs. . . . . and/or such assistants as may be selected by them, to perform the following procedure(s):

. . . . . .

. . . . . .

. . . . . .

2. Drs. . . . . have explained the purpose of this study and I understand the routine of the procedure outlined above.

. . . .

Witness Signature of Subject

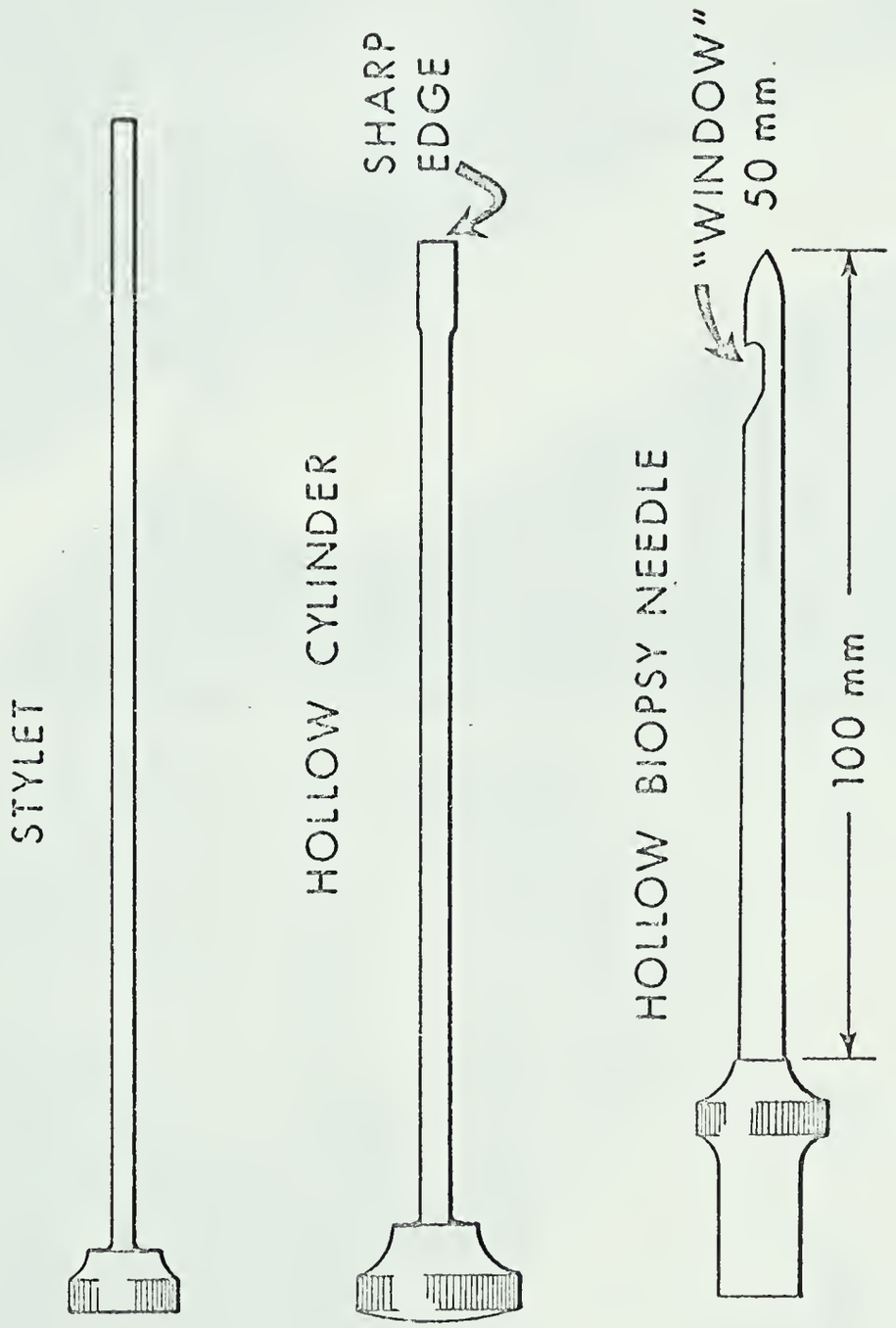
If the subject is unable to sign or is under 21 years of age, complete the following:

The subject is a minor (. . . . . years of age).
or
The subject is unable to sign because . . . . . .

As the closest relative or legal guardian I hereby sign on his/her behalf:

. . . . . .

Witness Signature Relationship



2. MUSCLE BIOPSY NEEDLE

4. MUSCLE GLYCOGEN CONTENT IN GRAMS GLYCOGEN/100 GRAMS WET MUSCLE FOR THE SUBMAXIMAL TEST

Subject	Percentage of total work time at which sample was taken							
	0(Initial)	24	35	50	65	76	100(Fatigue)	Resting
				SEDENTARY				
W.G.	1.10	0.81	1.16		0.99	0.76	0.87	1.10
D.M.	1.25	1.22		1.10		1.04	0.66	1.02
B.L.	1.07		0.78		0.49		0.59	0.67
G.M.	1.98*	0.78*	1.76*	1.39*	1.22*	1.36*	1.21*	1.10*
R.S.	0.89		0.71		0.50		0.26	0.32
W.H.	0.98*		1.11*		0.92*		1.29*	1.69*
J.S.	0.82		0.95		0.36		0.14*	0.67
				SEMI-ACTIVE				
G.W.	0.56*	1.12		0.86		0.72	0.76	0.54
R.P.	1.82	1.61	1.78	1.67	1.59	1.07	0.77	1.08
S.D.	1.45	1.23		1.26		1.01	0.50	0.28
K.M.	1.50	1.60	1.35		1.02	0.92	1.02	0.68
B.Y.	1.42		1.23		1.16		0.76	1.02
J.G.	1.11		0.99		0.37		0.42	0.61
B.P.	1.15	1.05		0.89		0.80	0.81	0.39
G.H.	0.92	1.13		0.95		0.71	0.40	0.53
J.R.	0.86	0.73		0.63		0.55	0.67	0.45
M.B.	1.04			1.15			0.78	0.92
A.T.	0.85		0.74		0.40		0.31	0.33

..... continued

Table 4 continued ...

Subject	Percentage of total work time at which sample was taken							
	0(Initial)	24	35	50	65	76	100(Fatigue)	Resting
				ACTIVE PRE-TRAINING				
R.L.	1.19	0.62		0.75		0.47	0.50	0.40
T.T.	1.47	1.07		0.50		0.96*	0.34	0.53
W.R.	0.74		0.66		0.42		0.38	0.32
A.M.	1.09	1.04		0.69		0.51	0.56	0.77
D.C.	1.42		0.73		0.41		0.90*	0.78
L.A.	1.84	1.47	1.21		1.41	1.32	1.15	1.55
B.S.	1.02	0.79		0.27		0.31	0.13	0.52
K.S.	1.44	1.13		1.01		0.85	0.48	1.07
H.R.	0.92*		0.99*		1.01*		0.99*	0.89*
B.J.	1.11	0.95		0.89		0.74	0.62	0.29
D.J.	0.86	0.66		0.70		0.91*	0.83*	0.63
				ACTIVE POST-TRAINING				
R.L.	0.59*			0.94			0.65	0.56
T.T.	0.51*	0.55*	1.16		1.10	0.96	0.87	0.74
W.R.	0.75	0.94		0.67		0.65	0.23	0.16
A.M.	0.85	0.51		0.71*		0.47	0.32	0.55
D.C.	1.47			1.42			0.82	0.72
L.A.	1.76		1.95		1.22		1.06	1.05
B.S.	0.44*		1.03		0.51		1.18*	0.69
K.S.	1.47			1.21			1.01	1.12
H.R.	1.24		0.70		0.68		0.70	0.67
B.J.	1.94	0.87		0.71		0.30	0.24	0.25
D.J.	1.19	1.44		1.09		0.78	1.41	0.30

* Muscle glycogen samples unacceptable.

5. MUSCLE GLYCOGEN CONTENT IN GRAMS GLYCOGEN/100 GRAMS WET MUSCLE FOR THE MAXIMAL TEST

Subject	Initial	Fatigue	Final Resting
	SEDENTARY		
W.G.	1.06	0.68	0.77
D.M.	2.38*	2.04*	2.13*
B.L.	0.80	0.27	0.66
G.M.	2.26*	2.17*	1.16*
R.S.	0.82	0.72	0.98
W.H.	0.33*	0.76*	0.49*
J.S.	0.74	0.69	0.81
	SEMI-ACTIVE		
G.W.	1.17	1.06	0.83
R.P.	1.41	1.76	0.68
S.D.	0.68	0.66	0.62
K.M.	1.90	1.80	1.25
B.Y.	1.09	0.99	1.15
J.G.	0.92	0.35	1.07
B.P.	1.34	1.22	1.00
G.H.	.67*	0.98	1.11
J.R.	1.47	1.25	1.16
M.B.	0.94	0.92	0.72
A.T.	1.50	0.96	0.77
	ACTIVE PRE-TRAINING		
R.L.	1.21	1.15	0.77
T.T.	1.35	1.16	1.08
W.R.	1.62	1.46	1.46
A.M.	1.03	0.60	0.58
D.C.	0.87	1.16	1.14
L.A.	1.36	1.51	0.92
B.S.	0.84	0.96	0.94
K.S.	1.54	1.29	1.46
H.R.	0.65*	0.61*	0.95*
B.J.	0.23*	0.83	0.57
D.J.	0.81	0.90	0.77

..... continued

Table 5 continued ...

Subject	Initial	Fatigue	Final Resting
		ACTIVE POST-TRAINING	
R.L.	1.63	1.25	1.00
T.T.	1.31	1.19	1.52
W.R.	1.58	0.91	1.29
A.M.	1.45	0.92	0.71
D.C.	1.28	0.74	0.82
L.A.	1.52	1.41	1.47
B.S.	1.58	.95	1.52
K.S.	2.43	2.22	2.10
H.R.	0.77	0.40	0.85
B.J.	2.03	1.65	0.68
D.J.	2.13	1.92	1.37

* Muscle glycogen samples unacceptable.

APPENDIX C

1. ANALYSIS OF VARIANCE BETWEEN GROUPS ON INITIAL VALUES FOR SUBMAXIMAL TEST - GLYCOGEN

Source	S.S.	D.F.	M.S.	F	Critical F
Between	0.291	3	0.097	1.12*	8.62
Within	3.165	29	0.109		
Total	3.456	32			

* Not significant at 0.05 level.

2. ANALYSIS OF VARIANCE BETWEEN GROUPS ON FATIGUE VALUES FOR SUBMAXIMAL TEST - GLYCOGEN

Source	S.S.	D.F.	M.S.	F	Critical F
Between	0.210	3	0.070	1.29*	8.62
Within	2.618	29	0.090		
Total	2.828	32			

* Not significant at 0.05 level.

3. ANALYSIS OF VARIANCE BETWEEN GROUPS ON FINAL RESTING VALUES FOR SUBMAXIMAL TEST - GLYCOGEN

Source	S.S.	D.F.	M.S.	F	Critical F
Between	0.087	3	0.029	3.59*	8.61
Within	3.441	33	0.104		
Total	3.528	36			

* Not significant at 0.05 level.

4. ANALYSIS OF VARIANCE BETWEEN GROUPS ON INITIAL VALUES FOR MAXIMAL TEST - GLYCOGEN

Source	S.S.	D.F.	M.S.	F	Critical F
Between	2.011	3	0.670	5.04*	2.92
Within	3.997	30	0.133		
Total	6.008	33			

* Significant at 0.05 level.

5. ANALYSIS OF VARIANCE BETWEEN GROUPS ON FATIGUE VALUES FOR MAXIMAL TEST - GLYCOGEN

Source	S.S.	D.F.	M.S.	F	Critical F
Between	1.210	3	0.403	2.34*	2.90
Within	5.516	32	0.172		
Total	6.726	35			

* Not significant at 0.05 level.

6. ANALYSIS OF VARIANCE BETWEEN GROUPS ON FINAL RESTING VALUES FOR MAXIMAL TEST - GLYCOGEN

Source	S.S.	D.F.	M.S.	F	Critical F
Between	0.826	3	0.275	2.37*	2.90
Within	3.697	32	0.116		
Total	4.523	35			

* Not significant at 0.05 level.

7. ANALYSIS OF VARIANCE FOR SEDENTARY GROUP ON SUBMAXIMAL TEST - GLYCOGEN

Source	S.S.	D.F.	M.S.	F	Critical F
Between	0.436	2	0.218	3.41*	3.98
Within	0.701	11	0.064		
Total	1.137	13			

* Not significant at 0.05 level.

8. ANALYSIS OF VARIANCE FOR SEMI-ACTIVE GROUP ON SUBMAXIMAL TEST - GLYCOGEN

Source	S.S.	D.F.	M.S.	F	Critical F
Between	2.274	2	1.137	15.16*	3.33
Within	2.182	29	0.075		
Total	4.456	31			

* Significant at 0.05 level.

9. ANALYSIS OF VARIANCE FOR ACTIVE PRE-TRAINING GROUP ON SUBMAXIMAL TEST - GLYCOGEN

Source	S.S.	D.F.	M.S.	F	Critical F
Between	2.476	2	1.238	10.58*	3.38
Within	2.913	25	0.117		
Total	5.389	27			

* Significant at 0.05 level.

10. ANALYSIS OF VARIANCE FOR ACTIVE POST-TRAINING GROUP ON SUBMAXIMAL TEST - GLYCOGEN

Source	S.S.	D.F.	M.S.	F	Critical F
Between	2.605	2	1.303	9.87*	3.37
Within	3.440	26	0.132		
Total	6.045	28			

* Significant at 0.05 level.

11. ANALYSIS OF VARIANCE FOR SEDENTARY GROUP ON MAXIMAL TEST - GLYCOGEN

Source	S.S.	D.F.	M.S.	F	Critical F
Between	0.141	2	0.071	1.25*	4.26
Within	0.510	9	0.057		
Total	0.651	11			

* Not significant at 0.05 level.

12. ANALYSIS OF VARIANCE FOR SEMI-ACTIVE GROUP ON MAXIMAL TEST - GLYCOGEN

Source	S.S.	D.F.	M.S.	F	Critical F
Between	0.471	2	0.236	1.98*	3.33
Within	3.463	29	0.119		
Total	3.934	31			

* Not significant at 0.05 level.

13. ANALYSIS OF VARIANCE FOR ACTIVE PRE-TRAINING GROUP ON MAXIMAL TEST - GLYCOGEN

Source	S.S.	D.F.	M.S.	F	Critical F
Between	0.214	2	0.107	1.16*	3.37
Within	2.403	26	0.092		
Total	2.617	28			

* Not significant at 0.05 level.

14. ANALYSIS OF VARIANCE FOR ACTIVE POST-TRAINING GROUP ON MAXIMAL TEST - GLYCOGEN

Source	S.S.	D.F.	M.S.	F	Critical F
Between	1.112	2	0.556	2.44*	3.32
Within	6.851	30	0.228		
Total	7.963	32			

* Not significant at 0.05 level.

15. ANALYSIS OF VARIANCE ON MVO_2 FOR ACTIVE PRE-TRAINING AND POST-TRAINING GROUPS

Source	S.S.	D.F.	M.S.	F	Critical F
Between	1.00	1	1.00	1.72*	4.35
Within	11.63	20	0.58		
Total	12.63	21			

* Not significant at 0.05 level.